DATA EVALUATION RECORD

TRIFLUMEZOPYRIM

STUDY TYPE: 28 Day RANGE FINDING -RAT

(NON-GUIDELINE)

MRID 49382157

Prepared for
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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Task 6-169

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DATA EVALUATION RECORD[1]

STUDY TYPE: 28-Day Range Finding Dietary - Rat; OCSPP 870.3050.

PC CODE: 129210 **DP BARCODE:** D432127

TEST MATERIAL (PURITY): Triflumezopyrim (99% a.i.)

SYNONYMS: DPX RAB55, 2,4-Dioxo-1-(5-pyrimidinylmethyl)-3-(3-(trifluoromethyl)-phenyl)-2H-pyrido(1,2-a)pyrimidinium inner salt

CITATION: Anand, S.S. (2013); DPX-RAB55 Technical: Repeated-dose oral toxicity 28-day feeding study in rats. DuPont Haskell Laboratory, Newark, Delaware, USA. Testing Facility Report No.: DuPont-33430. March 28, 2013. MRID 49382157.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, Delaware 19898

EXECUTIVE SUMMARY:

In a 28-day feeding study (MRID 49382157), triflumezopyrim was administered to male and female Crl:CD(SD) rats (5 animals/sex/dose) at concentrations of 0, 200, 800, 4000, or 20,000 ppm. Due to body weight loss, the 20,000 concentration was reduced to 10,000 ppm starting on Day 3. The mean daily intakes at these concentrations for males were 0, 17, 65, 309, and 653 mg/kg bw/day, respectively. The mean daily intakes for females were 0, 16, 64, 317, and 627 mg/kg bw/day, respectively. Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, hematology, coagulation, clinical chemistry, urinalysis, biochemical and thyroid hormonal parameters, gross pathology, and organ weights, and histopathology.

No treatment-related mortality or clinical signs were seen. Treatment-related decreases in absolute body weight were observed. In males, decreases at 4000 ppm and 10,000 ppm were 11% and 27%, respectively. In females, body weight was decreased 17% at 10,000 ppm. Decreases in body weight gain, food consumption, and food efficiency were also noted at 4000 and 10,000 ppm. No treatment-related changes were noted in the 200 or 800 ppm groups.

Decreases in red cell mass parameters (red blood cell, hemoglobin, and hematocrit) were present in male and female rats at 10,000 ppm and in females at 4000 ppm. These red cell changes were not considered to be treatment-related due to the minimal magnitude of effect (generally less than 10%), the absence of other effects in the hematopoietic system, and no treatment-related effects in these parameters after longer exposure in the 90-day study (MRID

[1] This DER was generated by modifying the study summary in a Tier II document (MRID 49382105).

49382162). No treatment-related effects were observed on coagulation, clinical chemistry, or urinalysis parameters.

Treatment-related effects on accessory sex organs (ASO) were seen in males at ≥4000 ppm. The effects included decreased fluid in the seminal vesicles and coagulating glands (10,000 ppm), decreased accessory sex organ weights (≥4000 ppm), and small seminal vesicles (10,000 ppm). Due to the lack of corroborative histopathological findings in the ASO tissues and the lack of any ASO organ weight or microscopic finding in the 90-day feeding study in rats (at concentrations up to 6000 ppm), the decreased ASO fluid was not considered to be adverse.

Treatment-related increases in liver weights, discoloration of the liver, and hepatocellular hypertrophy were observed in males and/or females at ≥4000 ppm. Treatment related effects on hepatic enzyme parameters (P450, UDPGT, and beta-oxidation) were observed in male and female rats at 4000 and/or 10,000 ppm. In the absence of other effects indicative of liver injury (e.g., alterations in relevant clinical chemistry parameters and histopathology), changes in liver weights and hypertrophy were considered to be non-adverse, adaptive responses to exposure to a xenobiotic.

No test substance-induced alterations were observed in T_3, T_4, or TSH concentrations.

The No-Observed-Adverse-Effect-Level (NOAEL) in Sprague Dawley rats was 800 ppm (65 mg/kg bw/day).

The Lowest-Observed-Effect-Level (LOAEL) was 4000 ppm (309 mg/kg bw/day in males) based on decreases in absolute body weight in males.

This 28-day oral toxicity study in the rat is classified as **Acceptable/Non-Guideline**.

COMPLIANCE: This range-finding study is not required to be in compliance with Good Laboratory Practice (GLP) Standards; however, work was conducted in a GLP-compliant facility following Standard Operating Procedures.

I. MATERIALS AND METHODS

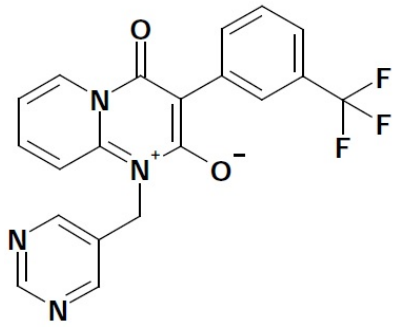
A. MATERIALS

1. Test material:

Triflumezopyrim technical

Lot/Batch #:	RAB55-028
Purity:	99%
Description:	Bright yellow solid
CAS #:	1263133-33-0
Stability of test compound:	Analyses confirmed that test material was stable in feed for at least 22 days at room temperature, was distributed uniformly in the feed, and was present in the feed at targeted concentrations.

Structure:



2. Vehicle:

Untreated diet

3. Test animals:

Species:	Rat
Strain:	Crl:CD(SD)
Age at initial dosing:	Approximately 50 days old
Weight at initial dosing:	198.3–233.5 g for males; 160.8–192.0 g for females
Source:	Males: Charles River Laboratories, Inc., Raleigh, NC. Females: Charles River Laboratories, Inc., Kingston, NY
Acclimation period:	7 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet (#5002), *ad libitum*. During the test period, test substance was incorporated into the feed of all animals except negative controls.
Water:	Tap water, *ad libitum*
Housing:	Animals were housed two or three per cage in solid-bottom caging with bedding mixed with enrichment.

4. Environmental conditions:

Temperature:	20–26°C
Humidity:	30–70%
Air changes:	At least 10 changes/hour
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS:

1. In-life dates: Start: 9/10/2011; End 09/09/2011

2. Animal assignment: Groups of 5 animals/sex/concentration were administered concentrations (0, 200, 800, 4000, or 20,000/10,000 ppm) of triflumezopyrim in feed daily for 30 and 31 days in males and females, respectively. The 20,000 ppm concentration was reduced to 10,000 ppm on Test Day 3 due to marked body weight loss in both sexes at the 20000 ppm concentration. Males received 0, 17, 65, 309, and 653 mg/kg bw/day,

respectively, and females received 0, 16, 64, 317, and 627 mg/kg bw/day, respectively. The 20,000/10,000 ppm concentration was selected to produce a limit dose exposure (approximately 1000 mg/kg bw/day) and some toxicity. The other concentrations were selected to assess a dose response for any observed effects and to establish a no-observed-adverse-effect level (NOAEL). Animals were assigned to dose groups by computerized, stratified randomization so that there were no statistically significant differences among group body weight means within a sex. A negative control group received untreated diet.

Table 1. Study Design: 28-Day Feeding Study in Rats

Males				Females			
Group No.	**Animals/ Group**	**Concentration in diet (ppm)[a]**	**Mean Daily Intake (mg/kg bw/day)**	**Group No.**	**Animals Group**	**Concentration in diet (ppm)[a]**	**Mean Daily Intake (mg/kg bw/day)**
1	5	0 (control)	0 (control)	1	5	0 (control)	0 (control)
2	5	200	17	2	5	200	16
3	5	800	65	3	5	800	64
4	5	4000	309	4	5	4000	317
5	5	10,000[b]	653	5	5	10,000[b]	627

[a] Weight/weight concentration of test substance
[b] Due to body weight decreases, the initial concentration of 20,000 ppm was reduced to 10,000 ppm starting Day 3.

3. **Diet preparation and Analysis:** The test substance was added to the rodent diet and thoroughly mixed for a period of time that is adequate to ensure homogenous distribution. Control diets were mixed for the same period of time. Diets were stored at room temperature until used. The stability, homogeneity, and concentration of triflumezopyrim in the dietary mixtures were checked by analysis using HPLC with ultraviolet (UV) detection at beginning of study. The concentration of triflumezopyrim in the dietary mixtures was also checked at the end of the study. The test substance was at target concentrations (± 7.5% of nominal), homogeneous (RSDs ≤3%) throughout the feed, and was stable (200-20,000 ppm) for up to 22 days at room temperature. Based on this information, it can be concluded that the animals received the targeted dietary concentrations of test substance during the study.

4. **Statistics:**

Table 2. Statistical Analyses: 28-Day Feeding Study in Rats

Parameter	**Preliminary test**	**If preliminary test was not significant**	**If preliminary test was significant**
Body weight Body weight gain Food consumption Food efficiency Clinical pathology[a] Organ weight	Levene's test for homogeneity and Shapiro-Wilk test for normality	One-way analysis of variance followed by Dunnett's test	Transforms of the data to achieve normality and variance homogeneity were used. The order of transforms attempted was log, square-root, and rank-order. If the log and square-root transforms failed, the rank-order was used.
Cytochrome P450 (total and isozymes) β-Oxidation UDPGT Hormone levels	Levene's test for homogeneity and Shapiro-Wilk test for normality[b]	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's test

[a] When an individual observation was recorded as being less than a certain value, calculations were performed on half the recorded value. For example, if bilirubin was reported as <0.10, 0.05 was used for any calculations performed with that data. When an individual observation was recorded as being greater than a certain value, calculations were performed on the

recorded value. For example, if specific gravity was reported as >1.083, 1.083 was used for any calculations performed with that data.

[b] If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.

C. METHODS

1. **Observations:** Animals were observed at least twice daily for mortality and morbidity and for signs of abnormal behavior and appearance. On days when they were weighed, each animal was individually handled, examined for abnormal behavior and appearance, and subjected to detailed clinical observations.

2. **Body weights:** All animals were weighed once per week. Additional body weights were collected during the first 2 weeks of exposure for animal welfare reasons, due to body weight loss at the high concentration.

3. **Food consumption and compound intake:** During the test period, the amount of food consumed by each rat over the weekly weighing interval was determined by weighing the feeder at the beginning and end of the interval and subtracting the final weight from the initial weight divided by the number of rats in the cage. Food efficiency and daily intake were calculated from food consumption and body weight data. Since the high concentration was reduced to 10,000 ppm on test Day 3, food consumption, food efficiency, and mean daily intake were calculated for intervals Day 0–3 and Day 3–7.

4. **Hematology, Clinical Chemistry and Urinalysis:** Blood and urine samples were collected from all fasted animals approximately 4 weeks after initiation of the study, on the day of necropsy. At sacrifice, blood, bone marrow, and urine were collected. Hematology, clinical chemistry, coagulation, bone marrow smears, and urine analysis were all performed on the samples.

   The following hematology parameters were determined: red blood cell count; red cell distribution width; hemoglobin; absolute reticulocyte count; hematocrit; platelet count; mean corpuscular (cell) volume; white blood cell count; mean corpuscular (cell) hemoglobin; differential white blood cell count; mean corpuscular (cell) hemoglobin concentration; prothrombin time; and activated partial thromboplastin time.

   The following clinical chemistry parameters were determined: aspartate aminotransferase, alanine aminotransferase activities, sorbitol dehydrogenase, and alkaline phosphatase activities; total bilirubin; urea nitrogen; creatinine; cholesterol; triglycerides; glucose, total protein; albumin; calcium; globulin; inorganic phosphorus; sodium; potassium; chloride; and bile acids.

   The following parameters were determined: quality; ketone; color; bilirubin; clarity; presence of blood; urobilinogen; specific gravity; protein; pH; and microscopic urine sediment examination.

5. **Thyroid hormone evaluations:** Blood was collected from non-fasted male and female rats from each group, on test Day 23. Serum was prepared and stored between -60 and -80°C until analyzed for hormone concentrations of triiodothyronine (T_3), thyroxine (T_4), and thyroid stimulating hormone (TSH).

6. **Biochemistry/mechanistic parameters:** At sacrifice, ~ 7 grams of the liver from each animal was snap frozen in liquid nitrogen and stored between -60 and -80°C. Hepatic microsomes and peroxisome were prepared by differential centrifugation. The microsomal suspensions were analyzed for UDPGT activity, total cytochrome P450 content, and quantification of individual cytochrome P450 isozymes 1A1, 1A2, 2B1/2, 2E1, 3A2, and 4A1/2/3 activities. The protein content of the microsomes and peroxisomes was determined before analysis by the Biorad method.

7. **Sacrifice and pathology:** At termination, animals were sacrificed by isoflurane anesthesia and exsanguination. Gross examinations were performed on all animals. Organs that were weighed are listed in Table 3. Group mean organ weight values and organ weight ratios (% body weight and % brain weight) were calculated. Tissues collected from animals receiving the highest dose (20,000/10,000 ppm) and control (0 ppm) were processed to slides and evaluated microscopically (Table 3). Gross lesions and suspected target tissues (male and female livers; male seminal vesicles and coagulating glands), as determined by examination of the control and high dose animals, were processed to slides and examined microscopically for all animals.

Table 3. 28-Day feeding study in rats: Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Brain (three sections)[a]	X	X
Spleen	X	X
Heart	X	X
Liver (two lobes)	X	X
Kidneys[b]	X	X
Oesophagus		X
Adrenal glands[b]	X	X
Duodenum		X
Jejunum		X
Ileum		X
Cecum		X
Colon		X
Rectum		X
Salivary glands[b, c]		X
Pancreas		X
Skin		X
Trachea		X
Nose (four sections)		X
Larynx/pharynx		X
Thymus	X	X
Mesenteric lymph node		X
Mandibular lymph node[b]		X
Bone marrow[d]		X
Peyer's patches[e]		X
Thyroid gland		X
Parathyroid glands[b]		X
Eyes[b, f]		X
Testes[b, f]	X	X
Epididymides[b, f]	X	X

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Prostate		X
Seminal vesicles (with coagulating glands)[b]		X
Ovaries (including oviducts)[b]	X	X
Uterus (including cervix)	X	X
Vagina		X
Mammary glands (females)		X
Accessory sex organs[g]	X	
Stomach		X
Pituitary gland		X
Lungs (two lobes)		X
Spinal cord (three levels)[h]		X
Sciatic nerve		X
Optic nerves[b]		X
Skeletal muscle[i]		X
Femur/knee joint		X
Sternum		X
Aorta		X
Urinary bladder		X
Gross observations[j]		X

[a] Brain includes cerebrum, cerebellum, midbrain, and medulla/pons.
[b] Both left and right organs.
[c] Salivary glands included mandibular, sublingual, and parotid glands.
[d] Bone marrow was collected with the femur and sternum.
[e] Peyer's patches were collected with the intestines.
[f] Fixed in Davidson's solution.
[g] Prostate + seminal vesicles + coagulating glands with their fluids
[h] Spinal cord includes cervical, mid-thoracic, and lumbar sections.
[I] Biceps femoris skeletal muscle.
[j]Gross observations made at necropsy for which histopathology was not appropriate (*e.g.*, fluid, ruffled fur) were generally not collected

II. RESULTS AND DISCUSSION:

A. OBSERVATIONS:

1. Clinical signs of toxicity: No treatment-related clinical signs of toxicity were observed at any dose.

2. Mortality: All animals survived to scheduled sacrifice.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

At the initiation of the study, high-dose animals (20,000 ppm) lost body weight over test Days 0-3. Therefore, this dose was reduced to 10,000 ppm for the remainder of the study. Males at 10,000 ppm exhibited adverse, treatment-related decreases in body weight parameters compared to controls. Statistically significant decreases in mean body weights and body weight gains were noted at 10,000 ppm throughout the test period (Tables 4 and 5). Final mean body weights (Day 28) and overall (Day 0–28) body weight gains at 10,000 ppm were 27% and 57%, respectively, lower than control (both

statistically significant). When the dietary concentration was lowered to 10,000 ppm, animals gained weight, but the level of body weight gain was less than in controls (statistically significant over most weekly intervals). Absolute bodyweights were also adversely decreased in males at 4000 ppm (↓11%) with body weight gain decreased by 24% as compared to controls.

Females at 10,000 ppm exhibited treatment-related decreases in body weight parameters compared to controls. Statistically significantly lower mean body weights (all intervals) and body weight gains (numerous intervals) were noted at 10,000 ppm. Final (Day 28) mean body weights and overall (Day 0-28) body weight gains at 10,000 ppm were 17% and 58%, respectively, lower than control (both statistically significant). There were no adverse body weight changes at doses ≤4000 ppm. The sporadic statistical significance noted in body weight gains at ≤4000 ppm was not considered treatment related as there was no consistent dose response and there were no associated significant decreases in body weights compared to control.

Table 4. 28-Day feeding study in rats: Body weights (g) ±SD

	0 ppm	**200 ppm**	**800 ppm**	**4000 ppm**	**10,000 ppm**
Males					
	0 mg/kg/day	**17 mg/kg/day**	**65 mg/kg/day**	**309 mg/kg/day**	**653 mg/kg/day**
Day 28	416.2±12.4	406.8±16.8	383.1±22.7	370.6±24.1[a] (-11%)	303.4±21.4[a] (-27%)
Females					
	0 mg/kg/day	**16 mg/kg/day**	**64 mg/kg/day**	**317 mg/kg/day**	**627 mg/kg/day**
Day 28	243.7±11.6	238.8±16.1	238.1±20.1	230.5±18.4	201.7±10.4[a] (-17%)

[a] Significantly different from control by the Dunnett 2 sided test criteria, p <0.05.

Table 5. 28-Day feeding study in rats: Body weight gain (g) ±SD

	0 ppm	**200 ppm**	**800 ppm**	**4000 ppm**	**10,000 ppm**
Males					
	0 mg/kg/day	**17 mg/kg/day**	**65 mg/kg/day**	**309 mg/kg/day**	**653 mg/kg/day**
Overall body weight gain Day 0–28	201.9±8.2	193.5±14.3	172.6±18.4[a]	152.8±16.8[a]	87.8±20.2[a] (-56%)
Females					
	0 mg/kg/day	**16 mg/kg/day**	**64 mg/kg/day**	**317 mg/kg/day**	**627 mg/kg/day**
Overall body weight gain Day 0–28	69.5±11.0	67.9±14.6	63.3±13.0	55.7±11.3	29.1±8.5[a] (-58%)

[a] Significantly different from control by the Dunnett 2 sided test criteria, p <0.05.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY:

Males administered 10,000 ppm exhibited statistically significantly lower mean food consumption throughout the test period. Overall (Days 0–28) food consumption and food efficiency at 10,000 ppm were 36% and 33%, respectively lower (both statistically significant) compared to control (Table 6). At 4000 ppm, food consumption was significantly lower at all intervals except Days 7–14, and food efficiency was significantly lower only at Days 14–21 and 21–28 (Table 7).

Females administered 20,000/10,000 ppm exhibited decreases in nutritional parameters compared to controls. Statistically significantly lower mean food consumption was noted throughout the test period. Overall (Days 0-28) food consumption and food efficiency at 20,000/10,000 ppm were 36% and 35%, respectively lower (both statistically significant) compared to control. Statistically significant changes noted in the other groups were not considered test substance-related, as there was no time or dose response or there were no significant decreases in body weights.

Table 6. 28-Day Feeding Study in Rats: Food consumption (g/animal/day) ±SD

	0 ppm	**200 ppm**	**800 ppm**	**4000 ppm**	**10,000 ppm**
Males					
	0 mg/kg/day	**17 mg/kg/day**	**65 mg/kg/day**	**309 mg/kg/day**	**653 mg/kg/day**
Food consumption, Days 0–28	24.9±0.0	25.0±1.4	23.1±0.4[a]	22.1±0.2[a] (-24%)	16.0±1.2[a] (-36%)
Females					
	0 mg/kg/day	**16 mg/kg/day**	**64 mg/kg/day**	**317 mg/kg/day**	**627 mg/kg/day**
Food consumption, Days 0–28	17.0±0.2	16.5±0.6	16.0±0.1	15.7±1.5	10.8±0.7[a] (-36%)

[a] Significantly different from control by the Dunnett non-parametric 2 sided test criteria, $p < 0.05$.

Table 7. 28-Day Feeding Study in Rats: Food efficiency ±SD

	0 ppm	**200 ppm**	**800 ppm**	**4000 ppm**	**10,000 ppm**
Males					
	0 mg/kg/day	**17 mg/kg/day**	**65 mg/kg/day**	**309 mg/kg/day**	**653 mg/kg/day**
Food efficiency, Days 0–28	0.289±0.012	0.277±0.008	0.267±0.028	**0.246±0.026**	**0.195±0.042[a] (-33%)**
Females					
	0 mg/kg/day	**16 mg/kg/day**	**64 mg/kg/day**	**317 mg/kg/day**	**627 mg/kg/day**
Food efficiency, Days 0–28	0.146±0.023	0.147±0.030	0.141±0.029	0.128±0.029	0.096±0.024[a] (-35%)

[a] Significantly different from control by the Dunnett 2 sided test criteria, $p < 0.05$.

D. CLINICAL PATHOLOGY:

1. **Hematology:** Red cell mass parameters (red blood cell count [RBC], hemoglobin [HGB] and hematocrit [HCT]) were minimally decreased in male rats fed 10,000 ppm (8–12%) and female rats fed 4000 or 10,000 ppm (8–11%) after 4 weeks of test substance exposure (Table 8). The decreases in red cell mass were associated with an increase in red cell distribution width (RDW) in these same groups (7–10%). All these changes were statistically significant compared to controls. These changes were not considered adverse given the small magnitude of the changes indicating the changes are not biologically significant. Furthermore, in 90-day studies in rats with triflumezopyrim (MRID 49382161 and MRID 49382162), no adverse effects on red cell mass parameters were observed in males or females fed dietary concentrations of up to 6000 ppm.

Table 8. 28-Day feeding study in rats: hematology findings ±SD

	0 ppm	200 ppm	800 ppm	4000 ppm	10,000 ppm
Males					
	0 mg/kg/day	**17 mg/kg/day**	**65 mg/kg/day**	**309 mg/kg/day**	**653 mg/kg/day**
RBC[a] (× 10^6/µL)	7.80±0.24	7.65±0.33	7.59±0.15	7.74±0.17	7.16±0.37[b] (-8%)
HGB[c] (g/dL)	14.7±0.2	14.4±0.6	14.5±0.6	14.7±0.8	13.4±1.2[b] (-9%)
HCT[d] (%)	45.6±1.0	44.1±1.5	44.1±2.1	43.8±1.6	40.1±2.2[b] (-12%)
RDW[e] (%)	12.0±0.2	11.8±0.3	12.0±0.3	11.9±0.3	13.1±1.0[f]
ARET[g] (× 10^3/µL)	274.8±31.9	256.8±11.1	271.2±27.0	254.9±29.4	298.4±53.9
Females					
	0 mg/kg/day	**16 mg/kg/day**	**64 mg/kg/day**	**317 mg/kg/day**	**627 mg/kg/day**
RBC (× 10^6/µL)	7.88±0.35	7.47±0.18	7.51±0.30	7.25±0.23[b] (-8%)	7.24±0.4[b] (-8%)
HGB (g/dL)	14.9±0.6	14.1±0.2	14.4±0.4	13.7±0.7[b] (-9%)	13.5±0.4[b] (-9%)
HCT (%)	44.3±1.8	42.6±0.6	42.8±1.1	40.1±1.9[b] (-9%)	39.5±2.1[b] (-11%)
RDW (%)	10.9±0.3	11.3±0.4	11.5±0.6	11.7±0.4[b]	12.0±0.3[b]
ARET (× 10^3/µL)	196.4±11.5	256.7±39.9	250.5±69.5	232.8±30.5	203.9±23.2

[a] Red blood cells
[b] Significantly different from control by the Dunnett 2-sided test criteria, $p < 0.05$.
[c] Hemoglobin
[d] Hematocrit
[e] Red cell distribution width
[f] Significantly different from control by the Dunnett non-parametric 2-sided test criteria, $p < 0.05$.
[g] Absolute reticulocyte count

2. **Clinical chemistry:** There were no adverse, test substance-related effects on clinical chemistry. A few statistically significant differences were observed; however, these changes were not considered adverse.

Cholesterol (CHOL) was increased in male and female rats fed 10,000 ppm (135% and 176% of control, respectively; statistically significant). Although the individual values for CHOL were higher than the concurrent control, most were within the 95% historical control range (38–86 mg/dL in males, 48–101 mg/dL in females), and there were no statistically significant changes in serum triglycerides. Small changes in serum cholesterol are frequent findings in toxicology studies in rats and are generally believed to represent minor effects on lipid metabolism that do not adversely affect the health of the animals. The increase in cholesterol in the 10,000 ppm male and female groups is likely test substance-related, but non-adverse.

Bilirubin (BILI) was minimally lower in female rats fed 4000 or 10,000 ppm (75% and 66% of control, respectively; statistically significant). Since increases, rather than decreases, in bilirubin are associated with biologically significant effects, these decreases in BILI were not considered to be adverse. Enzyme induction may enhance the metabolism and excretion of bilirubin, and total and individual cytochrome P450 enzymes were induced in the present study at ≥4000 ppm.

Blood urea nitrogen (BUN) was higher in male rats fed 10,000 ppm (152% of control). There were no correlative changes in serum creatinine or urinalysis parameters, nor in test substance-related microscopic changes in the kidneys in this group. In addition, no statistically significant changes in BUN occurred in female rats at any of the dietary concentrations tested. Therefore, the higher BUN in the 10,000 ppm male group was not considered adverse.

Glucose (GLUC) was elevated (statistically significant) in female rats fed ≥800 ppm; however, the changes were minimal in nature. Furthermore, there were no statistically significant or dose-related changes in GLUC in male rats at any of the dietary concentrations tested. Therefore, these changes in glucose in the treated female groups were most likely spurious and, based on their minimal nature, not considered adverse.

3. **Coagulation:** There were no treatment-related changes in coagulation parameters in male or female rats.

4. **Urinalysis:** There were no adverse changes in urine parameters.

A. THYROID HORMONE EVAULATIONS:

No treatment-related changes in thyroid hormones were observed (Table 9). In female rats, there were statistically significant increases in serum T_3 concentrations at 200 and 800 ppm (134% and 139% of control). These increases were considered spurious and not test substance-related, due to the lack of effects at the higher dose levels (4000 and 10,000 ppm) and the absence of changes in other thyroid parameters (serum T_4 and TSH, and thyroid histopathology) in either male or female rats or in serum T_3 concentrations in male rats.

B. BIOCHEMISTRY/MECHANISTIC PARAMETERS:

Triflumezopyrim caused increases in hepatic peroxisomal β-oxidation activity (4000 and 10,000 ppm male and females), UDPGT activity, and total cytochrome P450 enzyme content (10,000 ppm in males and 4000 and 10,000 ppm in females), and cytochrome P450 isozymes 1A2 and 2B1/2 (10,000 ppm in males and females) (Table 9).

The effects on hepatic enzymes at 4000 and 10,000 ppm were accompanied by statistically significant increases in relative (to final body weight) liver weight and liver hypertrophy. In the absence of clinical chemistry changes or anatomic pathology evidence of hepatic cellular injury, the changes in biochemical parameters were considered test substance related, but not adverse, and were consistent with an adaptive response of increased metabolism due to exposure to xenobiotics.

Table 9. 28-Day feeding study in rats: Thyroid and biochemistry parameters.

	0 ppm	200 ppm	800 ppm	4000 ppm	10,000 ppm
Males					
	0 mg/kg/day	**17 mg/kg/day**	**65 mg/kg/day**	**309 mg/kg/day**	**653 mg/kg/day**
T_4 (µg/dL)	5.0	5.0	5.6	4.5	4.1
T_3 (ng/dL)	56.3	59.5	58.2	50.5	63.7
TSH (ng/mL)	4.5	4.5	3.7	3.8	3.6
Total P450 (nmol/mg protein)	0.394	0.438	0.373	0.511	0.757[a]
UDP-GT (nmol/min-mg)	26.0	23.3	19.8	22.8	36.2[a]
Cytochrome P450 1A1	646,161	629,827	631,336	634,225	623,463
Cytochrome P450 1A2	448,229	461,577	474,247	521,162	663,065[a]
Cytochrome P450 2B1/2	472,339	460,134	437,633	603,400	973,100[a]
Cytochrome P450 2E1	593,949	590,480	586,881	598,967	563,764
Cytochrome P450 3A2	784,803	784,260	805,300	832,504	934,409
Cytochrome P450 4A1/2/3	833,971	908,538	824,448	896,277	854,212
β-oxidation rate (nmol/min-mg)	6.6	6.9	6.8	11.9[a]	18.0[a]
Females					
	0 mg/kg/day	**16 mg/kg/day**	**64 mg/kg/day**	**317 mg/kg/day**	**627 mg/kg/day**
T_4 (µg/dL)	3.4	4.0	4.0	2.0	2.9
T_3 (ng/dL)	56.9	76.1[a]	79.2[a]	60.1	54.8
TSH (ng/mL)	2.5	2.2	1.7	1.7	2.3
Total P450 (nmol/mg protein)	0.340	0.363	0.391	0.489[a]	0.567[a]
UDP-GT (nmol/min-mg)	14.5	15.8	15.2	20.6[a]	28.7[a]
Cytochrome P450 1A1	325,931	329,624	333,871	337,343	331,851
Cytochrome P450 1A2	487,262	393,411	438,625	547,299	680,740[a]
Cytochrome P450 2B1/2	500,078	513,029	510,338	524,158	687,169[a]
Cytochrome P450 2E1	520,428	524,605	523,119	527,704	502,538
Cytochrome P450 3A2	544,860	528,853	556,758	537,353	531,782
Cytochrome P450 4A1/2/3	928,950	922,184	1,001,147	1,040,470	1,049,993
β-oxidation rate (nmol/min-mg)	6.2	4.9	6.5	9.6[a]	14.0[a]

a Significantly different from control, $p < 0.05$.
Cytochrome P450 isozyme values units are intensity.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** Increased liver weights were observed in males and females at 4000 ppm and above (Table 10). In the 10,000 ppm males, mean absolute, mean relative % brain weight, and mean relative % body weight liver weights were increased 7, 12, and 49%, respectively, as compared to the control values. In the 4000 ppm males, these values were increased 9, 11, and 22%, respectively. The comparatively larger increases in % body weight values were attributed to the 11% and 29% decrease in final body weights in the 4000 and 10,000 ppm groups, respectively, and were the only changes that were statistically significant when compared to controls.

   In the 10,000 ppm females, mean absolute, mean relative % brain weight and mean relative % body weight liver weights were increased 14, 18, and 38%, respectively, as compared to the control values. In the 4000 ppm females, these values were increased 11, 12, and 18%, respectively. The statistically significant increases included the % body weight at 4000 ppm and all liver weight parameters at 10,000 ppm. As with males, decreased mean final body weights in the 4000 ppm (6%) and 10,000 ppm (17%) concentration groups contributed to the liver % body weight increases.

Decreased mean final body weights in males and females at 4000 and 10,000 ppm depressed the mean absolute and mean relative % brain weight while accentuating the mean relative % body weight. Microscopic hepatocellular hypertrophy in both sexes at 4000 ppm and above correlated with the increased liver weights.

In the 10,000 ppm males, mean absolute, mean relative % brain weight and mean relative % body weight ASO weights were decreased 54, 51, and 35%, respectively, as compared to the control values. In the 4000 ppm males, these values were decreased 21, 19, and 11%, respectively. All differences were statistically significant, except for the 4000 ppm mean relative % body weight ASO decrease.

The lower ASO weights in the 4000 and 10,000 ppm males appeared to be attributable to a lesser amount of fluid in the seminal vesicles (SVs) and coagulating glands (CGs). The only test substance-related microscopic changes in any of the ASOs was a minimal decrease, relative to controls, in the fluid content of the SVs and CGs of the 10,000 ppm males. Due to the lack of corroborating microscopic effects, the decrease in ASO fluid was not considered adverse.

Table 10. 28-Day Feeding Study in Rats: Mean terminal body and organ weights ±SD.

Parameter	0 ppm	200 ppm	800 ppm	4000 ppm	10,000 ppm
Males					
	0 mg/kg/day	**17 mg/kg/day**	**65 mg/kg/day**	**309 mg/kg/day**	**653 mg/kg/day**
Accessory sex organs (g)	2.581±0.16	2.597±0.16	2.380±0.34	2.050±0.19[b,c]	1.192±0.36[b,c]
Accessory sex organs/brain weight (%)	121.634±8.49	124.656±11.95	115.806±17.05	98.136±8.29[a,c]	59.187±18.28[a,c]
Accessory sex organs/terminal body weight	0.660±0.025	0.689±0.07	0.657 ±0.081	0.588±0.037[c]	0.430±0.133[b,c]
Liver (g)	11.175±1.06	10.793±0.82	10.604±0.57	12.165±1.04[c]	11.927±1.55[c]
Liver/brain weight (%)	525.718±40.35	517.733±51.10	515.042±16.47	582.225±43.62[c]	591.057±71.7[c]
Liver/terminal body weight (%)	2.859±0.26	2.852±0.13	2.932±0.12	3.493±0.25[a,c]	4.270±0.25[a,c]
Females					
	0 mg/kg/day	**16 mg/kg/day**	**64 mg/kg/day**	**317 mg/kg/day**	**627 mg/kg/day**
Liver (g)	6.312±0.40	6.745±0.37	6.928±0.59	7.009±0.77[c]	7.227±0.27[ac]
Liver/brain weight (%)	341.179±35.29	363.165±23.09	374.799±26.23	382.152±31.49[c]	401.910±26.01[a,c]
Liver/terminal body weight (%)	2.794±0.14	2.921±0.20	3.098±0.089	3.303±0.28[a,c]	3.868±0.21[a,c]

[a] Statistical Test: Dunnett 2 Sided p <0.05
[b] Statistical Test: Dunnett non-Parametric 2 Sided p <0.05
[c] Values were interpreted to be test substance-related.

2. **Gross pathology:** One of five males in the 10,000 ppm dietary concentration group was observed to have small seminal vesicles. The small seminal vesicles correlated with both the ASO organ weight effect in this group and the microscopic finding of decreased fluid in the SVs and CGs of this individual. Although the prostate in this rat was also described as small, it did not correlate with any microscopic finding and was, therefore, interpreted to be an incidental finding.

   At necropsy, four females, including one from the 800 ppm group and three from the 10,000 ppm group, had pale discoloration of the liver. In the three 10,000 ppm rats this observation correlated with minimal hepatocellular hypertrophy. In the 800 ppm rat and one 10,000 ppm female this observation correlated with minimal periportal fatty change.

All other gross observations were consistent with normal background lesions in rats of this age and strain.

3. **Histopathology:** In males, hepatocellular hypertrophy was observed in 0/5, 0/5, 0/5, 4/5, and 5/5 rats in the 0, 200, 800, 4000, and 10,000 ppm concentration groups, respectively. At 4000 ppm the hypertrophy was graded minimal (grade 1 of 4) in all affected rats. At 10,000 ppm, it was graded minimal in one rat and mild (grade 2) in the other four rats (Table 11). In females, hepatocellular hypertrophy was observed in 0/5, 0/5, 0/5, 1/5, and 3/5 rats in the 0, 200, 800, 4000, and 10,000 ppm concentration groups, respectively. All hypertrophy was graded as minimal. The hepatocellular hypertrophy was characterized by an increase in the size of centrilobular hepatocytes due to an increase in cytoplasmic volume. In mild cases, the hypertrophy also extended into the periportal hepatocytes. In both sexes, the hypertrophy correlated with increased mean liver weight parameters. In the three affected 10,000 ppm females, the hypertrophy correlated with pale discoloration of the liver. Exposure to xenobiotics commonly induces hepatic metabolic enzymes in laboratory animals. The hepatocellular hypertrophy observed in this 28-day feeding study, and the associated increased liver weights, were consistent with the non-adverse pharmacological induction of hepatic enzymes. The lack of an increase in serum liver enzymes or other histopathological observations supports the interpretation of these changes as adaptation rather than toxicity.

   Decreased fluid in the lumens of both the SVs and CGs were observed in 0/5, 0/5, 0/5, 0/5, and 4/5 rats in the 0, 200, 800, 4000, and 10,000 ppm concentration groups, respectively. The decreases were all graded as minimal. One 10,000 ppm rat was unaffected. The decrease in SV and CG fluid at 10,000 ppm correlated with the decrease in ASO weights at 4000 ppm and above (Table 12).

Table 11. 28-Day feeding study in rats: Incidence and severity of liver lesions in rats.

	0 ppm	200 ppm	800 ppm	4000 ppm	10,000 ppm
Males					
	0 mg/kg/day	**17 mg/kg/day**	**65 mg/kg/day**	**309 mg/kg/day**	**653 mg/kg/day**
Number of rats	5	5	5	5	5
Liver: Hepatocellular Hypertrophy	0	0	0	4	5
minimal	-	-	-	4	1
mild	-	-	-	-	4
Females					
	0 mg/kg/day	**16 mg/kg/day**	**64 mg/kg/day**	**317 mg/kg/day**	**627 mg/kg/day**
Number of rats	5	5	5	5	5
Liver: Hepatocellular Hypertrophy	0	0	0	1	3
minimal	-	-	-	1	3

Table 12. 28-Day feeding study in rats: Incidence of decreased fluid in the seminal vesicles and coagulating glands of male rats.

	0 ppm	200 ppm	800 ppm	4000 ppm	10,000 ppm
Males					
	0 mg/kg/day	**17 mg/kg/day**	**65 mg/kg/day**	**309 mg/kg/day**	**653 mg/kg/day**
Number of rats	5	5	5	5	5
SVs: Decreased Fluid, minimal	0	0	0	0	4
CGs: Decreased Fluid, minimal	0	0	0	0	4

III. CONCLUSION

A. INVESTIGATOR'S CONCLUSIONS:

The NOAEL for males was 4000 ppm (309 mg/kg bw/day). The LOAEL was 10,000 ppm (653 mg/kg bw/day) based on effects on body weight, nutritional, and red cell mass parameters. The NOAEL for females was 800 ppm (64 mg/kg bw/day). The LOAEL was 4000 ppm (317 mg/kg bw/day) based on effects on red cell mass parameters.

B. REVIEWER COMMENTS:

The reviewer agrees with the investigators conclusions that in the absence of other effects indicative of liver injury (e.g., alterations in relevant clinical chemistry parameters or histopathological findings), the observed mild to minimal liver hypertrophy was considered to be non-adverse, adaptive responses to exposure to a xenobiotic. The reviewer also agrees with the investigator's conclusion that the alterations in the seminal vesicles and the coagulating glands (e.g., decreased fluid and/or weights) at the high dose are not treatment-related due to the lack of corroborating observations. Furthermore, changes in the seminal vesicles or coagulating glands were not observed in a longer subchronic study in rats (MRID 49382162).

The reviewer does not agree with investigators on the decreases in red cell mass parameters. Although these changes were seen at the 4000 and 10,000 ppm, the magnitude of the changes were minimal (generally less than 10%), there were no other treatment related effects in the hematopoietic system (e.g., no effect on spleen), alterations in red cell mass parameters at 6000 ppm were within the historical control range, there are no corroborative histopathologic changes in this study, and similar effects were not seen at the same doses following a longer exposure period in the 90-day oral toxicity study in rats (MRID 49382162). Consequently, the reviewer does not agree with the NOAEL/LOAEL established for the females based on changes in the red cell mass parameters.

The No-Observed-Adverse-Effect-Level (NOAEL) for Sprague Dawley rats was 800 ppm (65 mg/kg bw/day).

The Lowest-Observed-Adverse-Effect-Level (LOAEL) was 4000 ppm (309 mg/kg bw/day) based on decreases in absolute body weight in males.

C. STUDY DEFICIENCIES:

None